REMARKS

The Office Action dated October 18, 2005, has been received and reviewed.

Claims 1-27 are currently pending and under consideration in the above-referenced application. Each of claims 1-27 stands rejected.

Reconsideration of the above-referenced application is respectfully requested.

FRET Quenching vs. Non-FRET Quenching

Non-FRET-Quenching

Lakowicz, Principles of Fluorescence Spectroscopy (2nd Edition) (hereinafter "Lakowicz"), a copy of which has already been provided to the Office, notes that non-fluorescence resonance energy transfer ("non-FRET") quenching can occur through a variety of mechanisms, none of which are considered to be non-radiative or radiationless energy transfer, or any type of resonance energy transfer. Non-FRET quenching of a fluorophore by another fluorophore or by a non-fluorophore quencher does not rely on resonance energy transfer. Instead of resonance energy transfer, non-FRET static or ground-state quenching involves formation of a non-fluorescent complex between a fluorophore and quencher that occurs while the fluorophore is in the ground state.

When non-FRET static or ground-state quenching occurs, light is absorbed by the fluorophore-quencher complex, but there is no fluorescence emission. Because of the ground-state interaction, there is, however, a change in the absorbance spectrum of the complex compared to unbound or free fluorophore. When the non-fluorescent complex is disrupted, non-FRET quenching no longer occurs, and the fluorophore is able to fluoresce. Thus, non-FRET quenching is an all-or-none phenomenon determined by whether the complex is associated (non-fluorescent) or dissociated (fluorescent). In contrast, resonance energy transfer (FRET) is a graded response that depends on the distance between donor and acceptor fluorophores.

Collisional or dynamic quenching is another form of non-FRET quenching that can occur during the excited state of the fluorophore. Quenching occurs when the excited fluorophore is deactivated by collision or diffusive encounter with a quencher molecule. This form of

quenching requires that the fluorophore be in the excited state. The contact with the quencher returns the fluorophore to the ground state without emission of a photon. The mechanism of dynamic quenching depends on the fluorophore-quencher pair, and can include electron transfer, spin-orbit coupling and intersystem crossing. Collisional quenching thus requires the absorption of a photon of light by the fluorophore, the consequent formation of the fluorophore excited state, and collision with a small molecule quencher during the excited state. The degree of collisional quenching is determined in large part by the concentration of quencher molecule, which determines the frequency of collisions with the excited-state fluorophore. In contrast to ground-state (static) quenching, there is typically no change in the absorption spectrum of the fluorophore with collisional quenching.

FRET-Quenching

Lakowicz teaches that non-radiative or radiationless *energy transfer* (as occurs in fluorescence resonance energy transfer ("FRET")), occurs between a donor and acceptor pair that are within a certain critical distance. In addition, the absorption (excitation) spectrum of the acceptor fluorophore must overlap with the emission spectrum of the donor fluorophore. The greater the spectral overlap, the more efficient the energy transfer between donor and acceptor. There is no emission of a photon during this process, thus the process is termed radiationless or non-radiative energy transfer. Energy transfer to a donor results in quenching of donor fluorescence, but an increase in fluorescence emission of the acceptor fluorophore.

In addition to spectral overlap, the degree of FRET-quenching between two fluorophores depends upon the distance between the two fluorophores. Specifically, the degree of FRET-quenching is the distance between the donor and acceptor fluorophores, raised to the inverse sixth power.

Claim Rejections—35 U.S.C. § 112, first paragraph

Claims 1-27 stand rejected under 35 U.S.C. § 112, first paragraph because the specification, while admittedly being enabling for the specific compounds disclosed, allegedly does not provide enablement for a scope covering all doubly labeled compounds that have at

least a part of their fluorescence quenched through ground state interaction. Applicants traverse this rejection for the following reasons.

<u>The specification is enabling for a biomolecular substrate including a molecular backbone with first and second fluorescent dyes that form a quenched intramolecular dye dimer.</u>

For a claimed genus, such as a biomolecular substrate including a molecular backbone with first and second fluorescent dyes that form a quenched intramolecular dimer, representative examples applicable to the genus as a whole are ordinarily enabling if one of skill in the art would expect the claimed genus could be used as disclosed in the specification without undue experimentation. (M.P.E.P. 2164.02). The fluorescent dyes and the molecular techniques for manipulating the biomolecular substrate and the molecular backbone are well known by those in the art. Additionally, those of skill in the art, without undue experimentation, would reasonably expect that a structure, according to the invention and including other biomolecular substrates of more or fewer amino acids, will work for a desired biomolecular substrate according to the claimed invention.

The art that is mentioned in the 35 U.S.C. § 112, first paragraph, rejection of claims 1-27, is actually very useful in distinguishing static (*e.g.*, ground-state) quenching from dynamic (*e.g.*, collisional or diffusive) quenching. In this regard, Lakos refers to experimental procedures that can be used to distinguish the contributions of static quenching (ground-state quenching) and collisional, or dynamic, quenching in a system where an external quencher is applied to a fluorescent protein. As an external quencher is applied, virtually all of the quenching detected will be dynamic quenching. The scenario set forth in Lakos does not apply to the case of an internally quenched system such as a double-labeled peptide where substantially all of the quenching is known to be static (occurring before excitation) because of the very high effective concentrations of the two dyes, the site-specific nature of labeling, and the ability to stoichiometrically label each site, as the substrates, systems, and their use, to which the claims of the above-referenced application are directed.

Indeed, the abstract of Lakos states:

> Quencher molecules produce two kinds of quenching: static and dynamic. Static quenching occurs due to encounter pair formation between quencher and fluorophore molecules, while dynamic quenching requires bimolecular collisions. <u>Unless one of the mechanisms can be neglected</u>, steady state quenching experiments cannot provide information on the contributions of the two processes. However, time-resolved experiments are sensitive only to the dynamic process, and thus provide selective information about the relative motion of the quencher and fluorophore.

In the intramolecular dye-dimer situation pertinent to each of claims 1-27, the dynamic quenching mechanism can be neglected because the labeling of a static system is highly controlled and designed to obtain essentially complete static quenching.

Sillen describes the contribution of static versus dynamic quenching in the calculation of average fluorescence lifetimes in systems containing multiple fluorophores where decay of fluorescence following pulse excitation is multiexponential due to a variety of processes including both static and dynamic quenching. The relative contributions are important to consider in studies of fluorescence lifetimes in complex biological molecules containing multiple fluorophores (typically amino acids such as tryptophan), but Sillen has no bearing on the fluorescence properties of the molecules recited in any of claims 1-27. Although each intramolecular dye-dimer system recited in claims 1-27 has more than one fluorophore, it is essentially a single fluorophore system because the behavior of only one of the dyes in the dye-dimer is being characterized at a time. As indicated above, substantially all of the fluorescence quenching in such an intramolecular dye-dimer system is caused by static, or ground-state, quenching because of the highly controlled nature of the labeling.

Schobel describes the use of a Cy5 and Cy5.5 FRET pair for use in fluorescence immunoassays. It has been asserted that Schobel provides evidence for the difficulty in predicting the extent of static quenching versus other types of quenching or the difficulty of determining or designing systems with high levels of ground-state interactions. The system described in Schobel is quite different and much more complex than the simple intramolecular dye-dimer system described in the above-referenced application. The immunoassay system described in Schobel is a complex system where multiple labeling sites are present on each

component of the assay system. Schobel shows that under conditions of low labeling (~1-2 molecules of dye per molecule of protein), ground-state dimers are minimized and binding of the two components can be readily measured by FRET.

In contrast, the above-referenced application describes systems, their components, and use thereof, in which fluorescent dyes are covalently attached at two specific sites in a substrate, such as a peptide, is described. The chemistry can be readily controlled to achieve stoichiometric site-specific labeling. The results are substrates, systems, and methods in which substantially all of the quenching that occurs is static quenching.

Schobel actually relies upon the known differences between static quenching and dynamic quenching to distinguish between the two. In this regard, Schobel discloses an experiment in which a deliberate attempt is made to incorporate high levels of dyes into each of the proteins in the disclosed assay system (Table 1) to show that the percentage of ground-state dye-dimers increases with high molar ratios of dye to protein. This is not unexpected because the fluorescent labels used in Schobel are relatively non-specific and can label any number of lysine residues in any given protein. If two lysines are located close in space on the protein being labeled, the chances of obtaining ground-state quenched dye-dimers are increased as the labeling stoichiometry increases. Importantly, Schobel shows that the presence of such dye-dimers can be readily monitored by a shift in the excitation or absorption spectra of the fluophores (Figs. 4 and 5), a phenomenon that is characteristic of static interactions. Because of this shift in the excitation (absorption) spectrum, the amount of monomer versus dimer in the several fluorescently labeled proteins that are disclosed in Schobel (Table 3) can be readily calculated.

The phenomenon of the shift in the excitation spectrum (which is identical to the absorption spectrum for most fluorophores) was described in the above-referenced application because it distinguishes static quenching from dynamic quenching. Thus, Schobel supports the position that the basis of the substrates, systems, and methods disclosed and claimed in the above-referenced application – dequenching of double-labeled substrate which occurs by disruption of ground-state interactions – can be readily verified in any given instance without having to do anything more sophisticated than measuring the excitation (absorption) spectrum of the double-labeled substrate.

In view of the teachings of Lakos, Sillen, and Schobel, it is apparent that results are not predictable when one attempts to design a system that operates on the principle of FRET quenching, while biomolecular substrates that have very predictable and efficient static (ground-state) interactions can be readily designed.

Therefore, because the specification of the above-referenced application, as admitted by the Office, is enabling for using the specific compounds disclosed for a biomolecular substrate including a molecular backbone with first and second fluorescent dyes that form a quenched intramolecular dimer, the specification is also enabling for one of skill in the art to make and use the claimed invention comprising other doubly labeled compounds including a biomolecular substrate with a molecular backbone that includes first and second fluorescent dyes that form a quenched intramolecular dimer.

<u>Following the analysis in *In re Wands*, the specification is enabling for the claimed invention.</u>

To satisfy the enablement requirement, a specification must teach those skilled in the art how to make and use the scope of the claimed invention without undue experimentation. *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997). Furthermore, simulated or prophetic examples are permitted in patent applications (M.P.E.P., § 608.01(p)(II)) and the use of prophetic examples may make a patent enabling. *Atlas Powder Co. v. E.I. DuPont di Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984).

When determining undue experimentation, the PTO and the courts look to the factors outlined in *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). The factors include 1) the quantity of experimentation necessary, 2) the amount of direction or guidance presented, 3) the presence or absence of working examples, 4) the nature of the invention, 5) the state of the prior art, 6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and 8) the breadth of the claims.

In *In re Wands*, the United States Court of Appeals, Federal Circuit (CAFC) reversed a rejection for lack of enablement for an application claiming monoclonal hybridomas which secrete specific antibodies. *Id.* at 740. The CAFC found the disclosure of the Wands patent enabling because there was a high level of skill in the monoclonal antibody art and, despite the

relative unpredictable nature of the technology, the patent disclosure provided guidance and real working examples of the invention. *Id.* at 738.

The CAFC recognized the complexity of the inventive technology but disagreed with the PTO, reasoning that the existing working examples, along with the specification, would allow one of ordinary skill in the art to make and use the invention. *Id.* at 740. The CAFC stated that a considerable amount of experimentation is permissible if it is reasonable with regards to the nature of the art or if the specification provides a reasonable amount of guidance. *Id.* at 737. The CAFC reasoned that the specification contained considerable direction and guidance on how to practice the claimed invention, presented working examples, that all the methods needed to practice the invention were well known and that there was a high level of skill in the art at the time the application was filed. *Id.*

Following the analysis from *In re Wands*, the specification of the instant invention also would have allowed one of skill in the art to make and use the claimed invention without undue experimentation. The specification discloses detailed laboratory protocols and guidance for the full scope of the claims, the referenced methods are well known by those of skill in the art, *i.e.* peptide synthesis and dye labeling are known in the art. Furthermore, working examples are disclosed using representative biomolecular substrates such as the targets of several protein kinases. Also, the level of skill in the art was high at the time of the invention.

More particularly, Example 1 of the specification details a working example of a double-labeled protein kinase substrate with a molecular backbone consisting of SEQ ID NO: 5. Example 1 outlines the necessary steps for synthesizing and purifying the substrate and the peptide molecular backbone along with instruction for conjugating the desired dyes to the peptide backbone. Also, Example 1 suggests the use of other double-labeled protein kinase substrates for assaying other protein kinases such as PKA, PKC, and CaM kinase II. Additionally, Example 1 discusses the phosphorylation-dependent changes in the optical properties of the double-labeled substrate. (*See*, FIGs. 5 through 9).

It is submitted that the specification and the disclosed working examples, would have enabled one of skill in the art to make and use double-labeled biomolecular substrates as recited in the claimed invention without undue experimentation. The disclosed methods were well

known and easily modifiable for use with biomolecular substrates and molecular backbones having more or fewer amino acids. Those of skill in the art, without undue experimentation or effort, would have been able to predict and measure the effects of other dyes and peptides within the scope of the claimed invention.

Therefore, the specification is enabling for the scope of the instant claims because, like in *In re Wands*, the methods and techniques were predictable and well known in the art at the time of the invention, the specification provided significant and detailed guidance and included real working examples applicable to the entire scope of the claimed invention.

For the reasons presented herein, removal of the rejection of claims 1-27 is respectfully requested.

Furthermore, as evidenced by the art upon which the Office has relied in its 35 U.S.C. § 112, first paragraph, rejection of claims 1-27, one of ordinary skill in the art would readily understand the difference between static (*e.g.,* ground state, etc.) quenching and dynamic (e.g., collisional quenching, and FRET quenching. As such, the Office cannot ignore the recitation of non-FRET or ground-state quenching or interactions recited in claims 1-27.

Claim rejections—35 U.S.C. § 102

Claims 1-2, 5-7, 9, 11, 15 and 21 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Odom, O.W., *et al.*, "An apparent conformational change in $tRNA^{Phe}$ that is associated with the peptidyl transferase reaction," Biochemie 69:925-38 (1987) (hereinafter "Odom"), and under 35 U.S.C. § 102(e) as allegedly being anticipated by Gildea *et al.* (U.S. Patent No. 6,485,901). Applicants traverse this rejection for the following reasons.

The Office asserts that:

> "[E]ven in [a] situation in which the donor-acceptor pair of dyes are designed for efficient FRET quenching (nearly total spectral overlap), there are ground state interactions (static effects) that lead to a part of the quenching that occurs. This would lead one to an expectation of an inherent presence of a ground state interaction that is responsible for at least a part of the quenching that occurs."

(Office Action, p. 9). However, a claim is anticipated under 35 U.S.C. § 102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single reference which qualifies as prior art under 35 U.S.C. § 102. *Verdegaal Brothers v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Likewise, the identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

With respect to inherency, M.P.E.P. § 2112 provides:

> The fact that a certain result or characteristic <u>may</u> occur or be present in the prior art is not sufficient to establish inherency of that result or characteristic. *In re Rijckaert,* 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) . . . 'To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill . . .'' *In re Robertson,* 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1991).

Independent claim 1 is drawn to a biomolecular substrate that includes a core molecular backbone and first and second dyes that are covalently attached to the core molecular backbone. When the biomolecular substrate is not covalently modified, the first and second dyes associate to form a quenched intramolecular dye dimer. Such quenching is effected at least in part through a non-FRET mechanism. When the biomolecular substrate is covalently modified without being cleaved, the first and second dyes dissociate, which results in dequenching of at least one of the dyes.

Independent claim 15 recites a method for assaying covalent biomolecular modification. The method of independent claim 15 includes providing a sample that includes a core molecular backbone, as well as first and second dyes covalently attached to the core molecular backbone. Non-FRET induced changes in fluorescent or absorbance characteristics of the biomolecular substrate may be quantified to determine whether or to the biomolecular substrate has been covalently modified.

Odom

Odom discloses a doubly labeled $tRNA^{Phe}$ for testing the *energy transfer* from the coumarin ring of DCCH attached at positions 16 or 20 to the fluorescein of FITC attached to acp^3U at position 47. Odom, p. 931. More particularly, Odom uses non-radiative energy transfer, or fluorescence resonance energy transfer (FRET), between two dyes located at positions in the tRNA molecules to detect a conformational change in the tRNA molecules occurring during peptidyl transfer. *Id.* A difference in fluorescence quantum yield was observed between deacylated $tRNA^{Phe}$ and the AcPhe-tRNA, indicating the distance between the dye changed during peptidyl transfer, which in turn indicates a conformational change in the tRNA molecule occurred. During the peptidyl transferase reaction, the deacylation of the ribosome-bound AcPhe-tRNA involves the cleavage of covalent bonds. First the peptidyl-tRNA is cleaved from the carboxyl end of a growing peptide chain and then peptide bond formation proceeds with the aminoacyl-tRNA. The covalent bond cleavage occurs as a result of nucleophilic attack by the lone pair of electrons on the amino nitrogen of the aminoacyl-tRNA on the carbonyl carbon that attaches the growing polypeptide chain to the peptidyl-tRNA molecule in the P site of the ribosome.

It is respectfully submitted that Odom does not disclose each and every element as set forth in independent claims 1 and 15. More particularly, Odom discloses covalent modification of deacylated $tRNA^{Phe}$ AcPhe-tRNA; specifically, the deacylation of the ribosome-bound AcPhe-tRNA by the peptidyl transferase reaction involves the cleavage of covalent bonds. Odom, page 937, first column. As such, Odom does not disclose a biomolecular substrate that is covalently modified without being cleaved. Therefore, independent claims 1 and 15 and those dependent therefrom are not anticipated by Odom.

Moreover, Odom does not disclose a fluorescence which is at least partially quenched through a non-FRET transfer mechanism. Those of ordinary skill in the art understand that changes in FRET can be caused by factors other than changes in distance between donor and acceptor, as such, the authors of Odom qualify their findings by stating that "[a]ll the results are consistent with but in themselves do not conclusively establish that tRNA undergoes a conformational change as well as movement during the peptidyl transferase reaction." Odom,

abstract, p. 925; p. 937, first column. The experiments of Odom do not expressly or inherently suggest to one skilled in the art of fluorescence or enzymology that using dye-pairs that stack and quench by ground-state interactions would be inherent extensions of the FRET dye-pair methods as used in Odom.

Additionally, it is respectfully affirmed that those of skill in the art recognize important differences between FRET and ground-state quenching. *See*, Lakowicz. Lakowicz states that "[f]luorophores can form nonfluorescent complexes with quenchers. This process is referred to as static quenching since it occurs in the ground state and does not rely on diffusion or molecular collisions." *Id.* at Chapter 1.4.A. Lakowicz also states that, for static quenching, the formation of a nonfluorescent complex between the fluorophore and quencher results in a complex that can absorb light, but that immediately returns to the ground state without emission of a photon. *Id.* at Chapter 8.3. A key feature of static quenching is that the fluorescence intensity is proportional to the fraction of total fluorophores that are not complexed, which is determined by the concentrations of fluorophore and quencher and their association constant. *Id.* Also, ground state complex formation will frequently result in perturbation of the absorption spectrum of the fluorophore. *Id.*

In contrast, FRET involves "transfer of the excited-state energy from the initially excited donor (D) to an acceptor (A)...The rate of energy transfer depends upon the extent of spectral overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the quantum yield of the donor, the relative orientation of the donor and acceptor dipoles, and the distance between the donor and acceptor molecules...The transfer efficiency can be determined by steady-state measurements of the extent of donor quenching due to the acceptor." *Id.* at Chapter 13, p. 1.

As such, it is respectfully submitted that, while quenching of the donor fluorophore does occur with FRET, the quenching does not involve formation of a complex between a fluorophore and a quencher as it does in the case of non-FRET quenching. Moreover, FRET does not result in perturbations of the absorption spectrum of the fluorophore as it does with non-FRET quenching.

Further, as the disclosure of Odom is limited to FRET quenching, and non-FRET quenching does not involve FRET, Odom does not inherently describe a biomolecular substrate

that is configured for non-FRET quenching or a method in which non-FRET quenching may occur.

Therefore, Odom does not anticipate either the biomolecular substrate of independent claim 1 or the assay method of independent claim 15. Accordingly, under 35 U.S.C. 102(b), the subject matter recited in both independent claim 1 and independent claim 15 is allowable over the subject matter described in Odom.

Each of claims 2, 5-7, 9, and 11 is allowable, among other reasons, for depending directly from claim 1, which is allowable.

<u>Gildea</u>

As previously noted, Independent claim 1 is drawn to a biomolecular substrate that includes a core molecular backbone and first and second dyes that are covalently attached to the core molecular backbone. Claim 1 also recites, in part, that when the biomolecular substrate is covalently modified, the second dye dissociates from the first fluorescent dye.

Independent claim 15 recites a method for assaying covalent biomolecular modification. The method of independent claim 15 includes providing a sample that includes a biomolecular substrate comprising a core molecular backbone, as well as first and second dyes covalently attached to the core molecular backbone. Also, the method of claim 15 teaches that when the biomolecular substrate is covalently modified, the second dye dissociates from the first fluorescent dye.

Gildea discloses a non-naturally occurring polyamide probe called a peptide nucleic acid (PNA) which can hybridize to DNA and RNA. Gildea, col. 5, lines 29-34. The PNA probe includes donor and acceptor moieties located at opposite ends of the probe. The PNA probe targets a complementary DNA or RNA sequence and, upon hybridization to the target sequence by hydrogen bonding, a detectable signal from at least one acceptor or donor moiety can be used to monitor or quantitate the hybridization event.

Notably, several passages in Gildea may cause confusion with regard to the photophysical mechanisms that are disclosed in Gildea and those recited in the claims of the above-referenced application. These passages are located at col. 1, lines 17-41, and col. 13, lines 14-40, if Gildea.

In particular, col. 1, lines 17-41, of Gildea provides that FRET is "also known as non-radiative energy transfer" and that non-FRET interactions are "also known as radiationless energy transfer." The statement that FRET is known as "non-radiative energy transfer" is true. However, this term is used synonymously by those of skill in the art with the term "radiationless energy transfer." The statement in Gildea that non-FRET quenching is "radiationless" is not true.

In this regard, Lakowicz notes that non-FRET quenching can occur through a variety of mechanisms, none of which is considered to be "radiationless energy transfer." Non-FRET static or ground-state quenching, involves formation of a non-fluorescent complex between a fluorophore and quencher that occurs while the fluorophore is in the ground state. It does not rely on diffusion or molecular collisions, and *there is no energy transfer*.

In contrast, Lakowicz teaches that dynamic or collisional quenching occurs when an excited fluorophore is deactivated by collision or diffusive encounter with a quencher molecule. This form of quenching requires that the fluorophore be in the excited state. The contact with the quencher returns the fluorophore to the ground state without emission of a photon. The mechanism of dynamic quenching depends on the fluorophore-quencher pair, and can include electron transfer, spin-orbit coupling and intersystem crossing.

It is respectfully submitted that Gildea does not disclose a biomolecular substrate that includes a core molecular backbone and first and second dyes that are covalently attached to the core molecular backbone. More particularly, the non-naturally occurring and artificially synthesized PNA probe of Gildea does not include a biomolecular substrate as recited by independent claims 1 and 15. Furthermore, Gildea only discloses nucleotide hydrogen bonding between the PNA probe and the target DNA or RNA and, as such, does not disclose covalent modification of a biomolecular substrate.

In addition, from the inaccuracies that have been identified with respect to the disclosure of Gildea (*i.e.,* that non-FRET interactions involve "radiationless energy transfer"), one of ordinary skill in the art couldn't be reasonably certain, without undue experimentation, that non-FRET interactions actually occur in the molecules that are described in Gildea.

Therefore, Gildea does not anticipate either the biomolecular substrate of independent claim 1 or the assay method of independent claim 15. Accordingly, under 35 U.S.C. 102(e), the

subject matter recited in both independent claim 1 and independent claim 15 is allowable over the subject matter described in Gildea.

Each of claims 2, 5-7, 9, and 11 is allowable, among other reasons, for depending directly from claim 1, which is allowable. Likewise, claim 21 is allowable, among other reasons, for depending directly from the allowable claim 15.

Claim rejections—35 U.S.C. § 103(a)

Claims 1-27 stand rejected under 35 U.S.C. § 103(a). Applicants respectfully traverse this rejection for the following reasons.

The standard for establishing and maintaining a rejection under 35 U.S.C. § 103(a) is set forth in M.P.E.P. § 706.02(j), which provides:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

<u>Blumenthal in view of Odom and Tyagi</u>

Claims 1-27 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Blumenthal in view of Odom and Tyagi (WO 97/39008 or U.S. Patent No. 6,150,097). For the following reasons, it is respectfully submitted that the teachings of Blumenthal, Odom, and Tyagi do not support a *prima facie* case of obviousness against any of claims 1-27.

Blumenthal teaches biomolecular substrates that include a single dye (acrylodan) for measuring changes in the conformation of a calmodulin-binding domain peptide when the peptide binds calmodulin. In one of the methods, the analyzed peptide included naturally occurring tryptophan residues and was employed as a FRET donor molecule, while the acrylodan acted as the FRET acceptor molecule. When calmodulin binds *noncovalently* to the peptide, a

conformational change in the peptide occurred, which decreased the distance between the tryptophan and the acrylodan and, thus, caused an increase in FRET quenching. Blumenthal does not include any teaching or suggestion of use of non-FRET quenching to determine whether or not a conformational change in the peptide occurred, or that non-FRET quenching may be used to determine whether a covalent, non-cleavage modification of a biomolecular substrate has occurred.

The teachings of Odom have been summarized above. Most notably, the teachings of Odom are limited to use of a dye pair in which changes in FRET quenching are indicative of conformational changes in $tRNA^{Phe}$ that may occur as a ribosome-bound AcPhe-tRNA is deacylated to produce a $tRNA^{Phe}$ by the peptidyl transferase reaction. Odom, p. 937, first column. During the peptidyl transferase reaction, deacylation of the ribosome-bound AcPhe-tRNA involves the cleavage of covalent bonds. First the peptidyl-tRNA is cleaved from the carboxyl end of a growing peptide chain and then peptide bond formation proceeds with the aminoacyl-tRNA. The covalent bond cleavage occurs as a result of nucleophilic attack by the lone pair of electrons on the amino nitrogen of the aminoacyl-tRNA on the carbonyl carbon that attaches the growing polypeptide chain to the peptidyl-tRNA molecule in the P site of the ribosome. Like Blumenthal, Odom does not include any teaching or suggestion of use of non-FRET quenching to determine whether or not a biomolecular substrate has been modified or, more specifically, that non-FRET quenching may be used to determine when a biomolecular substrate has been covalently modified but not cleaved.

Tyagi teaches the use of "Molecular Beacon" oligonucleotide probes to monitor binding of oligonucleotide probes labeled with two fluorescent dyes or a fluorophore and a quencher to target sequences in nucleic acids. While fluorescence of the fluorophore may be quenched when the quencher "touches" the fluorophore and, thus, ground state quenching may occur, the quenching is merely indicative of hydrogen bonding between nucleic acids, not of covalent modification of the labeled nucleic acid probe. As such, Tyagi lacks any teaching or suggestion of use of non-FRET quenching to determine whether or not a biomolecular substrate has been covalently modified without being cleaved.

None of Blumenthal, Odom, or Tyagi teaches or suggests a biomolecular substrate that is covalently modified without being cleaved, as recited by independent claim 1; a method in which a biomolecular substrate is covalently modified without being cleaved, as recited in independent claims 15, 25, and 27; or a kit including a biomolecular substrate and a dye that, when the biomolecular substrate is covalently modified without being cleaved, dissociates from another dye to reduce quenching by ground-state interactions between the dyes, as recited in independent claim 26.

In regards to claims 23 and 24, it is respectfully affirmed that independent claim 23 is allowable since none of Blumenthal, Odom, or Tyagi teaches or suggests a method for assaying protein kinase activity, let alone various aspects of such a method, including provision of a biomolecular substrate that includes a KID peptide sequence or a pair of molecules that, when the biomolecular substrate is not covalently modified, form an intermolecular dye dimer, but, when the biomolecular substrate is phosphorylated, dissociate to reduce quenching between the pair of molecules.

Claim 24 is allowable, among other reasons, for depending directly from claim 23, which is allowable.

Moreover, without improperly relying upon the disclosure of the above-referenced application, one of ordinary skill in the art wouldn't have been motivated to combine teachings from Blumenthal, Odom, and Tyagi in the manner that has been asserted. This is because none of the references provides any motivation for using non-FRET quenching techniques with a core molecular backbone to detect covalent changes in the core molecular backbone. More specifically, while it is acknowledged that tRNA molecules undergo a conformational change during the peptidyl transferase reaction, Odom actually dissuades from the asserted combination of teachings by indicating that the FRET-based fluorescence quenching results set forth therein "do not conclusively establish that tRNA undergoes a conformational change . . . during the peptidyl transferase reaction." Odom, Abstract. Due to this admitted unreliability, it does not appear that one of ordinary skill in the art would have any reason to supply a purportedly analogous technology (non-FRET quenching) to the methodology that is taught in Odom.

Therefore, Odom provides no motivation for one of ordinary skill in the art to use substitute non-FRET quenching techniques for the FRET quenching techniques disclosed therein.

For these reasons, the teachings of Blumenthal, Odom, and Tyagi do not support a *prima facie* case of obviousness against any of claims 1-27. Accordingly, it is respectfully submitted that, under 35 U.S.C. § 103(a), the subject matter recited in each of claims 1-27 is allowable over the teachings of Blumenthal, Odom, and Tyagi.

Macala, Schultz or Ventura in View of Blumenthal and Odom or Tyagi

Claims 1-27 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over L. J. MACALA et al., "Measurement of cAMP-Dependent Protein Kinase Activity Using a Fluorescent-Labeled Kemptide," Kidney International 1998, Vol. 54, Pages 1746-1750 (hereinafter "Macala"), U.S. Patent 5,580,747 to Schultz et al. (hereinafter "Schultz"), or C. VENTURA et al., "Phorbol Ester Regulation of Opioid Peptide Gene Expression in Myocardial Cells," The Journal of Biological Chemistry, 15 December 1995, Vol. 270, No. 50, Pages 30115-30120 (hereinafter "Ventura"), in view of Blumenthal and Odom or Tyagi.

Macala teaches a protein kinase assay that uses a protein kinase substrate labeled with *one fluorophore*. The Macala assay includes electrophoretically separating the phosphorylated product from the nonphosphorylated fluorescent substrate using agarose gels. Also, unlike the claimed invention, the Macala assay is not a homogenous assay that can be use for continuous, high throughput screening of enzyme activity, nor for use in measuring enzyme activity in living cells. As acknowledged by the Office, Macala does not teach or suggest a substrate having two dyes attached or a library of compounds.

Schultz teaches a fluorescent assay using a modified substrate labeled with a single dye. The Schultz assay requires the separation of the reaction products from the substrate by electrophoresis, chromatography, or extraction. Like Macala, this assay is not a homogenous assay that can be use for continuous, high throughput screening of enzyme activity, nor for use in measuring enzyme activity in living cells. As acknowledged by the Office, Schultz does not teach or suggest a substrate having two dyes attached or a library of compounds.

Ventura, likewise, teaches a single-labeled fluorescent protein kinase peptide substrate. The single-labeled peptide shows a *20% decrease* in fluorescence intensity when it is fully phosphorylated. Because the fluorescence change is measured as a decrease in intensity with a maximum change of 20%, it is difficult to measure low levels of phosphorylation. As acknowledged by the Office, Ventura does not teach or suggest a substrate having two dyes attached or a library of compounds.

Blumenthal teaches the use of acrylodan-labeled synthetic peptides to study calmodulin-peptide interactions. The peptides are based on the calmodulin-binding domain of myosin light chain kinase. The paper describes fluorescence studies using these peptides, including studies where binding of acrylodan labeled peptides to calmodulin were measured by changes in fluorescence intensity due to changes in solvent exposure of the acrylodan, changes in fluorescence anisotropy due to changes in rotational diffusion, and changes in fluorescence intensity due to FRET between the intrinsic tryptophan donor and the acrylodan acceptor. The *noncovalent* binding of the peptide to calmodulin induces helix formation in the peptide that brings the donor-acceptor pair within FRET distance, causing an increase in fluorescence emission of the acceptor. None of the fluorescence studies in Blumenthal involved covalent modification or measurement of protein kinase activity, nor did they involve ground state quenching of fluorescence.

The teachings of Odom and Tyagi have been summarized above. Most notably, and as discussed previously, neither Odom nor Tyagi teach or suggest a biomolecular substrate that is covalently modified *without being cleaved.*

With respect to the purported combination of teachings from Macala, Shultz, Ventura, Blumenthal, and Odom, it is respectfully submitted that none of these references teaches or suggests a biomolecular substrate that includes a core molecular backbone and a pair of dyes that undergo ground-state interactions prior to covalent modification of the backbone and dissociate from one another following covalent modification of the backbone without being cleaved. Moreover, none of these references teaches or suggests use of such a biomolecular substrate in an assay. Thus, none of these references teaches or suggests each and every element of any of claims 1-27.

In addition, since Macala, Shultz, Ventura, Blumenthal, and Odom, taken together, do not teach or suggest each and every element of any of claims 1-27, one of ordinary skill in the art would have no reason to expect the purported combination of teachings from these references to be successful.

Moreover, it is respectfully submitted that, without the benefit of hindsight that the claims of the above-referenced application provide to the Office, one of ordinary skill in the art wouldn't have been motivated to combine teachings from Macala, Shultz, or Ventura, with teachings from Blumenthal and Odom. This is because peptides are relatively unstructured in solution, as opposed to nucleic acids, such as those taught in Odom, which self-hybridize in solution to form hair-pin structures between complementary internal sequences. As such, one of ordinary skill in the art would have had no reason to expect that attaching a dye near each end of a peptide might generate a reagent that would be useful for monitoring non-cleavage, covalent modifications to a peptide by non-FRET quenching.

For these reasons, it is respectfully submitted that, without improperly relying upon the hindsight provided by the disclosure and claims of the above-referenced application, one of ordinary skill in the art would have had no reason to combine the teachings of Macala, Shultz, or Ventura with those of Blumenthal and Odom in the manner that has been asserted.

As for the asserted combination of teachings from Macala, Shultz, or Ventura, with teachings from Blumenthal and Tyagi, it is respectfully submitted that one of ordinary skill in the art would have had no reason to combine the teachings of these references. Of these references, only Tyagi teaches fluorescence quenching between a pair of dye molecules. The teachings of Tyagi are limited to reductions in fluorescence quenching as a single stranded probe nucleic acid hybridizes with another single stranded nucleic acid, *which is a noncovalent modification* of the probe nucleic acid, while Macala, Shultz, Ventura, and Blumenthal merely teach use of single or non-interacting fluorescent molecules on a core molecular substrate. None of Macala, Shultz, Ventura, or Blumenthal teaches or suggests that a pair of dyes may be used to indicate covalent changes to a core molecular substrate. Further, one of ordinary skill in the art wouldn't have been motivated to combine teachings from Tyagi with those of Macala, Shultz, Ventura, or

Blumenthal due to stark dissimilarities between the peptide substrates of Macala, Shultz, Ventura, and Blumental and the nucleotide substrates of Tyagi. In particular, peptides are relatively unstructured in solution, as opposed to nucleic acids, such as those taught in Tyagi, which self-hybridize in solution to form hair-pin structures between complementary internal sequences. As such, one of ordinary skill in the art would have had no reason to expect that attaching a dye near each end of a peptide might generate a reagent that would be useful for monitoring non-cleavage, covalent modifications to a peptide by non-FRET quenching.

Thus, without improperly relying upon the hindsight provided by the disclosure and claims of the above-referenced application, it is not seen how the teachings of any of these references could have provided one of ordinary skill in the art to use the dye pair of Tyagi to detect covalent modifications to a core molecular substrate.

As no combination of teachings from Macala, Shultz, or Ventura, with those of Blumenthal and Odom or Tyagi supports a *prima facie* case of obviousness under 35 U.S.C. § 103(a), it is respectfully submitted that, under 35 U.S.C. § 103(a), the subject matter recited in each of claims 1-27 is allowable over the teachings of these references.

CONCLUSION

It is respectfully submitted that each of claims 1-27 is allowable. An early notice of the allowability of each of these claims is respectfully solicited, as is an indication that the above-referenced application has been passed for issuance. If any issues preventing allowance of the above-referenced application remain which might be resolved by way of a telephone conference, the Office is kindly invited to contact the undersigned attorney.

Respectfully submitted,

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